ZOOLOGICAL SCIENCE 6: 675–680 (1989) 

# New Morphological Aspects of the Brush Cells in the Main Excretory Ducts of the Rat Submandibular Glands

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**ABSTRACT**—The brush cells in the main excretory ducts (MED) of rat submandibular glands were observed by electron microscopy.

Characterized by long and thick microvilli, brush cells are scattered throughout the epithelium. The fine structures of the brush cells in this study are basically similar to those described in earlier studies. However, several brush cells concentric whorls of rER comprising several lamellae were observed at the juxtanuclear portion. The presence of these concentric whorls of rER alludes to the cellular activity of the brush cells. Moreover, several intranuclear inclusions were found in the nuclei of almost all the brush cells observed. Intranuclear inclusions in the brush cells have not been reported elsewhere in other organs or tissues so far investigated. Therefore, it seems that the brush cells in the MED of rat submandibular glands are characterized by the presence of intranuclear inclusions. In contrast, neither whorls of rER nor intranuclear inclusions are present in the epithelial cells of the MED. Therefore, we can assume that the brush cells have a different function from that of the epithelial cells.

## INTRODUCTION

Rhodin and Dalhamn [1] once observed a new type of cell in the rat trachea which was characterized by long and thick microvilli on the luminal surface, and thus named them "brush cells". The peculiar structure of the microvilli allows for easy identification of the brush cells by electron microscopy. Besides microvilli, other characteristics of brush cells include (1) the extension of many bundles of filaments from the top of each microvilli to the supranuclear region, and (2) the presence of numerous vesicles and tubules between the bundles of filaments in the apical cytoplasm.

The brush cells are observed throughout the epithelia of the respiratory system, that is, from the respiratory mucosa of the nasal cavity [2] to the alveolar epithelium [3]. Further, these cells have been identified in the epithelia of the digestive tract, including in the main excretory duct of the salivary glands [4, 5], the stomach [6, 7], the gallbladder [8, 9] and the colon [10]. In the MED of the salivary glands, the brush cells are known by other names, either as "tuft cells" [5] or "dark cells" [4, 11]. Various hypotheses concerning the function of brush cells are discussed. However, because of a scanty number of brush cells in the relevant organs and tissues, the structural details of the brush cells in relation to their function have not yet been established. Therefore, in the present study, we examined the fine structure of the brush cells in the MED of submandibular glands in rats.

Accepted September 19, 1988
Received May 28, 1988

## MATERIALS AND METHODS

MED were obtained from ten adult (170–200 g) male Wistar rats. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.), and the submandibular glands were exposed, with the length of the MED extending distally about 1 cm from the hilus. The MED was then ligated at the end of its distal exposure, taking care to avoid applying unnecessary tension to it. The area from

the ligature to the hilus was removed and immersed in 2.5% cold buffered glutaraldehyde. The ducts were cut off near both ends, and the remains were cut into approximately 2 mm segments, kept in the same solution and used in this study. After 1 hr of fixation in glutaraldehyde, the specimens were rinsed in buffer and transferred to 1% cold buffered osmium tetroxide, where the tissue was allowed to post-fix for 2 hr. Phosphate buffer at pH 7.4 was used to buffer the fixatives. Following dehydration, the specimens were embedded in spurr resin and sectioned for electron microscopy on an ultramicrotome. Ultra-thin sections were placed on uncoated copper grids and stained with

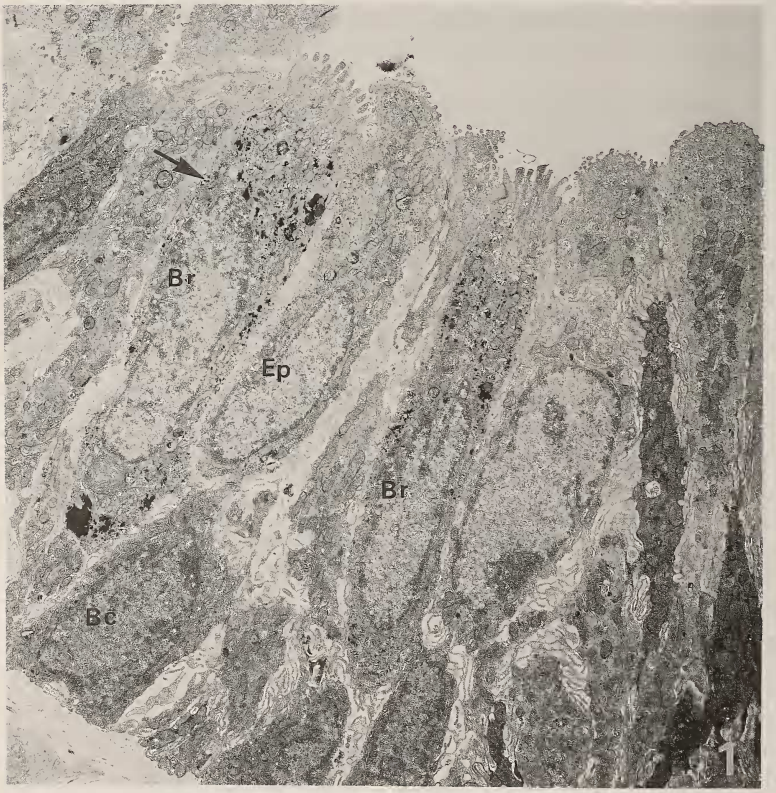



Fig. 1. MED in rat submandibular glands is composed of three cell types: epithelial cells (Ep), brush cells (Br) and basal cells (Bc). Brush cells have long, thick microvilli, bundles of filament extending from the top of microvilli to deep in the cytoplasm, many vesicles and glycogen granules. Concentric whorls of membranous structure and a well-developed rough surfaced endoplasmic reticulum (arrow) comprising several lamellae are seen around the irregular nucleus of the brush cell. ×6,000.

uranyl acetate [12] and lead citrate [13], and examined using a JEM 100B electron microscope.

## RESULTS

The main excretory ducts (MED) of the rat submandibular glands were composed of three cell types: epithelial cells, basal cells and brush cells (Fig. 1). The luminal surface of these cells frequently showed a bulbous expansion upon which short and stubby microvilli were present. The epithelial cells were predominant in number and made up of columnar cells. The nucleus was oval in shape containing a small nucleolus with a rounded appearance. Several basal cells, which had more electron-dense cytoplasm than other cell types, were situated under the epithelial cells and brush cells.

A total of 34 brush cells were examined in this study.

The brush cells were present between the epithelial cells and their microvilli were larger and more regularly spaced than those of the epithelial cells. The brush cells were easily distinguished from the surrounding epithelial cells in structure because of their long and regular arrangement microvilli. Desmosomes were present at the neck

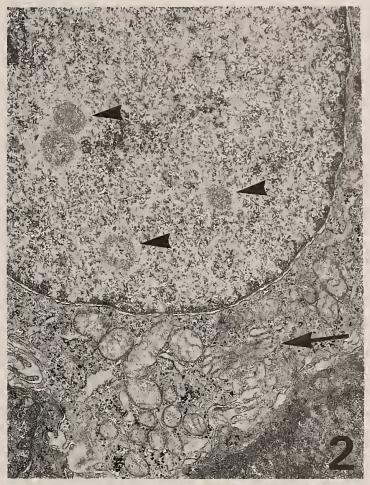
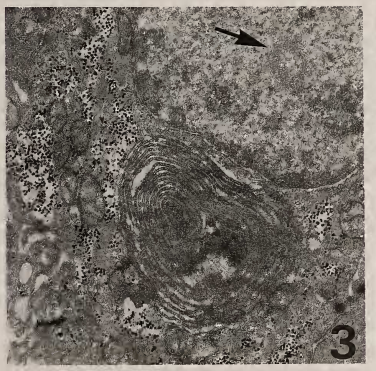



FIG. 2. Well-developed rough surfaced endoplasmic reticulum (arrow) is located in the basal portion, and several intranuclear inclusions (arrow heads) are seen in the nucleus of the brush cell. ×12,000.



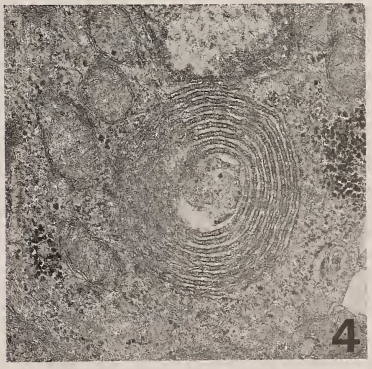




FIG. 3. Concentric whorls of membranous structure composed of several lamellae are seen in the basal portion of the brush cell. The intranuclear inclusion (arrow) is ring-shaped. ×18,000.

FIG. 4. Some ribosomes are attached to the inner- and outer-most lamellae of these membranous strucutures. ×37,000.

portion which was narrower than the basal area of the brush cells. The bundles of filaments extended from the core of the distal end of the microvilli to the supranuclear region. A few long, thin cytoplasmic processes without filaments were present on the lateral border. There were no cell organelles in the free zone beneath the microvilli of the brush cells, except for a large number of electron-lucent vesicles among the filaments. These vesicles, having different diameters, were either oval or tortuous in shape. Several vesicles contained dense granules. The nucleus, having a small, dense nucleolus, was rather irregular in shape and situated in the basal half of the brush cell. The nuclei of almost all brush cells contained several intranuclear inclusions (Figs. 1, 2 and 3). These intranuclear inclusions were comprised of fine granules and were oval or ring-shaped, and easily distinguished from surrounding nuclear matrices and nucleoli. They had a lower elecron-density than the nucleolus, and there was a halo between the nuclear matrix and the core of the intranuclear inclusion (Figs. 1, 2 and 3). No intranuclear inclusions were observed in any other cell type in the MED epithelium. Several lysosomes were located in the supranuclear region. Many mitochodria, rough surfaced endoplasmic reticulum (rER) in a short tubular form and numerous glycogen granules were scattered throughout the cytoplasm except in the apical part of the brush cells. Sometimes, rER with well-developed cisternae were observed in the cytoplasm near the nucleus (Figs. 1 and 2). In addition, concentric whorls of membranous structures composed of several lamellae were seen in the basal portion of some brush cells; such structures were seen in 5 out of 34 brush cells. Some ribosomes were attached to the inner- and outer-most lamellae of these structures (Figs. 1, 3 and 4). In contrast, epithelial cells had no whorls of membranous structures.

## DISCUSSION

The epithelium contains three cell types: epithelial cells, basal cells and brush cells, in the MED of the salivary glands [5, 11]. Differences in the structures of these three cell types make them easy to distinguish from one another. The fine structures of the cells examined in the present study are basically similar to those described in earlier studies [3, 5, 6, 8, 9]. The brush cells in the MED of salivary glands have also been called various other names, such as tuft cells [5, 14] or dark cells [11, 14]. Accordingly the structures of those cells correspond to the characteristics of brush cells in other organs and tissues. The ultrastructural findings in earlier studies were confirmed in the present investigation.

Many authors have reported that various shapes of intranuclear inclusions, composed of fine granules or bundles of filaments, are contained in the cells of many organs and tissues, for example, in pancreatic islets cells [15], endocrine cells of stomach [16], adrenocortical cells [17] and acinar cells of the human salivary labial glands [18]. However, intranuclear inclusions were not mentioned in the brush cells observed in earlier studies. Intranuclear inclusions similar in structure to those we found in the brush cells of the submandibular glands have been previously described by Tandler *et al.* [18], who regarded them as peculiar to the nuclei of acinar secretory cells of the human salivary labial glands. According to Boquist [15], in pancreatic islets cells, intranuclear inclusions might play some role in cellular activity, or in cell renewal and division. In acinar cells of the human salivary labial gland, they might be related to the secretory cycle of the mucous cells, since they were found only in immature cells [18]. However, almost all brush cells in the MED have several intranuclear inclusions, while epithelial cells in the MED have none. In addition, these structures have not been reported in brush cells in other organs and tissues. Therefore, the presence of intranuclear inclusions in the brush cell of the MED seems to be related to the function of these brush cells. Moreover, the brush cells in the MED are most likely not immature, because the brush cells observed contain rich filaments and well-developed rER, which are not typical features of immature cells. This suggests that the brush cells in the MED of rat submandibular glands are characterized by the presence of these structures.

The concentric whorls of membranous structure in the present study may be a kind of rER, since they have some ribosomes in the most inner and

outer lamellae. Nickerson [19] described the formation of concentric whorls of rER in adenocortical cells of the mongolian gerbil, and states that the function of these rER is the saving of rER in adenocortical cells. Brush cells containing concentric whorls of rER have not been reported in earlier studies. Many brush cells having no concentric whorls of rER in the MED of the submandibular glands contain well-developed rER. Following the administration of pilocarpine in the rat submandibular gland, the brush cells reached the state of hyperergasia, and the concentric whorls of rER were not observed (unpubl. data). Therefore, it seems that well-developed rER in brush cells is referable to concentric whorls of rER, and the formation of concentric whorls of rER may be related to cellular activity.

Several hypotheses have been put forward as to the function of brush cells, including that they might be reabsorptive [6, 20], secretory [21] and/or chemoreceptive [9, 22]. Due to the presence of large numbers of vesicles and tubules arranged in rows between bundles of filaments, brush cells have been considered to have a reabsorptive function [6]. However, Luciano and Reale [8] showed that electron microscopic observations with horseradish peroxidases injected into the lumen of the gallbladder did not reveal tracers within the vesicles and tubules of the brush cells. On the other hand, Qwarnström and Hand [14] observed that when horseradish peroxidase was injected into the lumen of the MED of the submandibular gland, the brush cells were found to contain horseradish peroxidase in the cytoplasmic matrix, but not in vesicles, other cell organelles or the nucleus. Therefore, a reabsorptive function for these cells has been ruled out. A secretory function for brush cells may also be disregarded since they do not show the typical morphology of secretory cells, which consists of a prominent Golgi apparatus and secretory granules. In addition, the features of microvilli and bundles of filaments in the brush cells of the rat submandibular gland after the administration of pilocarpine and isoproterenol were changed. However, the endocytosis and exocytosis in the brush cells has not been comfirmed. Following administration of those drugs, alterations in the components of saliva in the submandibular gland were investigated (unpubl. data). In the trachea, Luciano *et al.* [23] observed brush cells with a nerve ending. Therefore, they consider the function of brush cells to be chemoreceptive. Such neural connections were not confirmed in this study; however, the brush cells in the MED of the rat submandibular glands may indeed have a chemoreceptive function, since their morphological features, including characteristic microvilli, bundles of filaments, and large number of vesicles are similar to those of taste bud type 1 sensory cells [24].

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